

# Laboratory Evaluation of Face Paint Repellent Formulation Using Human Volunteers Under Three Climatic Regimens

RAJ K. GUPTA,<sup>1</sup> LOUIS C. RUTLEDGE,<sup>1</sup>  
AND ROBERT L. FROMMER<sup>2</sup>

J. Med. Entomol. 26(5): 468-470 (1989)

**ABSTRACT** A new combination of repellent with camouflage face paint was evaluated on volunteers against the mosquitoes *Aedes aegypti* (L.) and *Anopheles stephensi* (Liston) in an environmental chamber under three climatic regimens: tropical open, tropical forested, and hot dry environments. The duration of protection varied significantly among different climates. The camouflage face paint with diethylmethylbenzamide (deet) provided 95% protection from mosquitoes for 4 h, except under tropical open climate, where it provided only 2 h protection.

**KEY WORDS** Insecta, deet, *Aedes aegypti*, *Anopheles stephensi*

IN FIELD SITUATIONS, topical repellents are an economical and practical means of preventing the transmission of arthropod-borne disease. In a continuing effort to reduce manpower losses caused by arthropod-borne diseases, the Letterman Army Institute of Research is studying various means of incorporating repellents into topical lotions and creams to enhance effectiveness, persistence, and user acceptance.

The insect repellent currently issued by the U.S. Army (NSN 6840-00-753-4963) cannot be used with the currently issued camouflage face paint because the repellent is a solvent that causes the paint to run. For this reason soldiers using the face paint cannot be protected against biting insects. A new camouflage face paint-repellent formulation using *N,N*-diethyl-*m*-methylbenzamide (deet) is under development by the Army to replace the two incompatible items.

The camouflage face paint-repellent formulation contains three additives in addition to the face paint colors and the insect repellent. These additives are talc, ceresine wax, and a heavy, viscous mineral oil. A possible collateral effect of the additives would be sustained release of the repellent component (Reifenrath & Rutledge 1983, Mehr et al. 1985, Gupta et al. 1987). They also could enhance the cosmetic properties of the paint and user acceptance of the product.

The study evaluated the camouflage face paint-

repellent formulation against mosquitoes in a chamber under simulated tropical open, tropical forested, and hot dry environments.

## Materials and Methods

The insects used in this study were a yellow fever vector, *Aedes aegypti* (L.), obtained from the University of California at San Francisco, and a malaria vector, *Anopheles stephensi* (Liston), obtained from the Walter Reed Army Institute of Research, Washington, D.C. Mosquitoes were reared and maintained at  $27 \pm 3^\circ\text{C}$  and  $80 \pm 10\%$  RH under a 12:12 (L:D) photoperiod. Larvae were reared on a diet of floating catfish food (Continental Grain, Chicago). The adults were maintained on 10% sucrose solution. The mosquitoes used were nulliparous females between 5 and 15 d old.

The test method used was a modification of American Society for Testing and Materials (ASTM) standard E951-83, "Standard Methods for Laboratory Testing of Non-Commercial Mosquito Repellent Formulations on the Skin." The camouflage face paint-repellent formulation containing 20% deet was supplied in an olive drab compact containing four colors: loam, sand, white, and green. Compacts containing the camouflage face paints without repellent were tested for comparison. All colors of both compacts were tested for 12 h under three climatic regimens: (1) variable high humidity (tropical open),  $30^\circ\text{C}$  for entire 12 h with 78% RH for the first 6 h and 98% RH for the last 6 h; (2) constant high humidity (tropical forested),  $24^\circ\text{C}$  with 97.5% RH; and (3) hot dry,  $37^\circ\text{C}$  with 31% RH for the entire 12 h.

The paint-repellent formulation was applied evenly to the forearms (flexor region) of four volunteers. Each forearm was treated with one of the eight different color and repellent combinations of the formulation. One additional volunteer served

Opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the U.S. Department of the Army. Use of trade names does not constitute an official approval of the products mentioned.

Human subjects participating in this study gave free and informed voluntary consent.

<sup>1</sup> Letterman Army Institute of Research, Presidio of San Francisco, Calif. 94129.

<sup>2</sup> Natick Research, Development and Engineering Center, Natick, Mass. 01760.

**Table 1.** Mean number of bites ( $\bar{x} \pm SE$ ) by *Ae. aegypti* over 12-h period

Treatment	Climatic regimen		
	Constant high humidity	Variable high humidity	Hot
No treatment (control) <sup>a</sup>	8.5 $\pm$ 0.5a	14.0 $\pm$ 0.8a	14.4 $\pm$ 0.1a
Face paint with no deet <sup>b</sup>	10.5 $\pm$ 0.7a	12.6 $\pm$ 0.4a	14.2 $\pm$ 0.2a
Face paint with deet <sup>b</sup>	2.9 $\pm$ 0.7b	6.0 $\pm$ 0.8b	7.7 $\pm$ 0.6b

Means followed by the same letter in the same column are not significantly different.

<sup>a</sup> Mean number of bites recorded at seven time periods  $\times$  2 test days.

<sup>b</sup> Mean number of bites recorded at seven time periods  $\times$  2 test days  $\times$  four face paint colors. There were no significant differences among face paint colors.

as untreated control, testing one species of mosquitoes on each arm. A record was kept of the amount of each paint color applied on each arm. Volunteers were instructed not to rub, scratch, or wash the treated areas for the duration of the test period.

At the start of the test, a plastic cage (4 by 5 by 18 cm) containing 15 mosquitoes was bound to the forearm with Velcro tape, and a slide was withdrawn to expose the treated skin to the mosquitoes. The number of mosquitoes biting was recorded at the end of 90 s. The slide was then closed, and the test was repeated with the second species of mosquitoes. This procedure was repeated every 2 h for 12 h using a fresh cage of mosquitoes each time. Thus, seven tests of each species of mosquito were conducted on each formulation at 0, 2, 4, 6, 8, 10, and 12 h after application on the skin. The test volunteers stayed in the environmental chamber for the entire 12 h except for brief periods allowed for meals and use of the lavatory.

Assignment of the test subjects' arms to the eight treatments (four colors of formulation with deet and four with no deet) was at random on the first

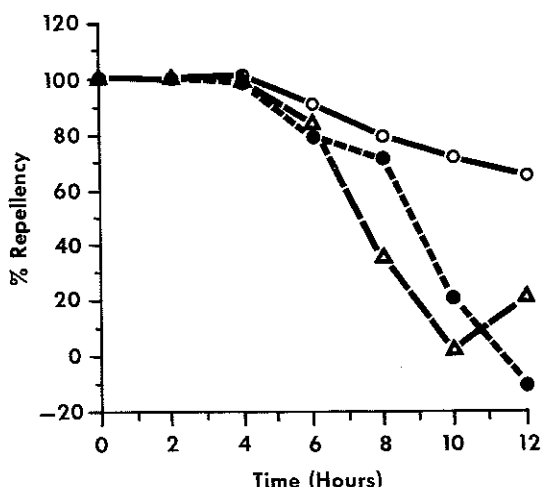
**Table 2.** Mean number of bites ( $\bar{x} \pm SE$ ) by *An. stephensi* over 12-h period

Treatment	Climatic regimen		
	Constant high humidity	Variable high humidity	Hot
No treatment (control) <sup>a</sup>	6.4 $\pm$ 0.8a	9.4 $\pm$ 0.1a	4.9 $\pm$ 0.6a
Face paint with no deet <sup>b</sup>	8.3 $\pm$ 0.5a	9.3 $\pm$ 0.3a	6.4 $\pm$ 0.9a
Face paint with deet <sup>b</sup>	0.9 $\pm$ 0.2b	2.9 $\pm$ 0.5b	1.7 $\pm$ 0.3b

Means followed by the same letter in the same column are not significantly different.

<sup>a</sup> Mean number of bites recorded at seven time periods  $\times$  2 test days.

<sup>b</sup> Mean number of bites recorded at seven time periods  $\times$  2 test days  $\times$  four face paint colors. There were no significant differences among face paint colors.



**Fig. 1.** Effectiveness of camouflage face paint-repellent formulation against *An. stephensi* in three climatic regimens. (○—○) constant high humidity; (△---△) variable high humidity; and (●----●) hot dry.

day of testing under each climatic regimen. The test was repeated with the same climatic regimen on the next day. The same arm received the same color of formulation with deet and with no deet on alternate days. The control was rotated in a random order.

**Statistical Analysis.** A four-way analysis of variance was done on the number of bites recorded using the Biomedical Data Program (BMDP2V) statistical package (Dixon et al. 1983) to check for significant differences among the formulation colors, climatic regimens, deet/no deet treatment, and time (hours after application of formulation) at 5% level of significance. The percentage of repellency was determined from the total number of bites on the control and treated test volunteers by Abbott's formula (Abbott 1925).

## Results and Discussion

The average ( $\pm SE$ ) weight of formulation colors with deet applied by the individual volunteers to their forearms (flexor region) was  $1.48 \pm 0.02$  mg/cm<sup>2</sup>. The weight of formulation color with no deet was  $1.07 \pm 0.01$  mg/cm<sup>2</sup>.

Tables 1 and 2 summarize the mean biting counts of *Ae. aegypti* and *An. stephensi*, during the 12-h test period under the three climatic regimens. In our study, we observed an overall mean biting rate of 8.30 bites/min for *Ae. aegypti* and 4.96 bites/min for *An. stephensi* in controls. Because the area of skin exposed in the test was 33 cm<sup>2</sup>, this is equivalent to a biting rate of 125 bites/min per forearm (500 cm<sup>2</sup> skin) for *Ae. aegypti* and 75 bites/min per forearm for *An. stephensi* for subjects using no deet. There were no significant differences between the number of bites on the control subjects

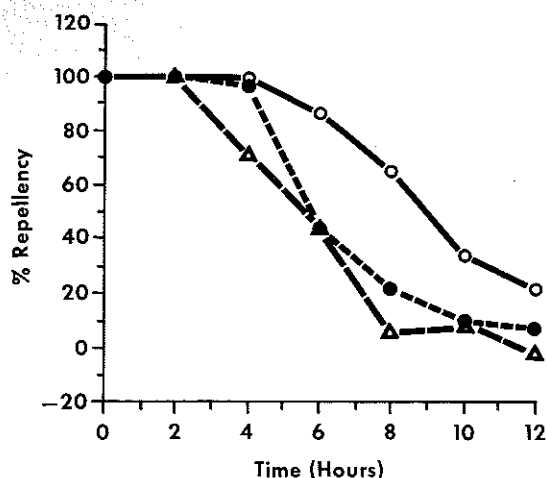


Fig. 2. Effectiveness of camouflage face paint-repellent formulation against *Ae. aegypti* in three climatic regimens. (○—○) constant high humidity; (●---●) variable high humidity; and (△---△) hot dry.

and the subjects using the four different camouflage face paint colors with no deet.

In the case of *An. stephensi*, the analysis of variance indicated that three main effects (climate, deet, and post-application time) and three interactions (post-application time × climate, deet × post-application time, and deet × post-application time × climate) were significant at the 5% level. The significant three-way interaction indicated that the effectiveness of the formulation with deet varied significantly with the type of climate and the time interval after application. The formulation with deet provided significantly greater protection than the formulation with no deet. The formulation with deet provided 95% or better protection for 4 h under all climatic regimens (Fig. 1).

The analysis of variance of the data for *Ae. aegypti* showed that the three main effects (climate, deet, and post-application time) and two interactions (post-application time × climate and deet × post-application time × climate) were significant at the 5% level. The significant three-way interaction indicated that the effectiveness of the formulation with deet varied significantly with the type of climate and the time interval after application. The volunteers using the formulation with no deet received as many bites as did the untreated controls, indicating that the formulation with no deet does not provide any protection against mosquito bites. The formulation with deet provided

significantly greater protection than the one with no deet. The formulation with the deet provided 95% or greater protection for 4 h under constant high humidity and variable high humidity as compared with only 2 h under the hot climatic regimen (Fig. 2).

Although the formulation with deet provided substantial protection for 12 h, it was not more than 95% effective for more than 4 h. Under the hot dry climatic regimen, both species were attracted to the formulation with deet. This effect occurred most often in the late hours of testing when the residue on the skin was presumably low. The attractancy of low residues also has been observed in other laboratory (Z. A. Mehr & L.C.R., unpublished data) and field studies (Gupta et al. 1987).

The camouflage face paint with deet provided 95% or greater protection for 4 h under various climatic regimens tested except under the hot dry climatic regimen. Combining deet in camouflage face paint is an idea that may reduce noncombat casualties, but its protection is relatively short under the severe climatic regimens tested. However, the effectiveness of a camouflage face paint-repellent formulation with deet is still to be evaluated under field conditions for protection and troop acceptability. In the past, deet has provided longer protection under field conditions than under the climatic conditions used in this study.

#### References Cited

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265-267.
- Dixon, W. J., M. B. Brown, L. Engleman, J. W. Fran, M. A. Hill, R. I. Jennrich & J. D. Toporek. 1983. BMDP statistical software. University of California Press, Berkeley.
- Gupta, R. K., A. W. Sweeney, L. C. Rutledge, R. D. Cooper, S. P. Frances & D. R. Westrom. 1987. Effectiveness of controlled-release personal-use arthropod repellents and permethrin impregnated clothing in the field. *J. Am. Mosq. Control Assoc.* 3: 556-560.
- Mehr, Z. A., L. C. Rutledge, E. L. Morales, V. E. Meixsell & D. W. Korte. 1985. Laboratory evaluation of controlled-release insect repellent formulations. *J. Am. Mosq. Control Assoc.* 1: 143-147.
- Reifenrath, W. G. & L. C. Rutledge. 1983. Evaluation of mosquito repellent formulations. *J. Pharm. Sci.* 72: 169-173.

Received for publication 7 September 1988; accepted 10 January 1989.